LEADING ARTICLE

Gut and mind

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Obesity is a growing epidemic, causally associated with a number of serious medical conditions, including diabetes mellitus, coronary heart disease, and several cancers. The gut hormones ghrelin and peptide YY are secreted from the gut in response to changes to nutritional status. While food intake is stimulated by ghrelin, it is inhibited by peptide YY. The discovery, anatomy, and physiology of ghrelin and peptide YY are discussed, focusing on the adaptive changes in diseases such as obesity and anorexia nervosa. Ghrelin and PYY are important therapeutic targets in the quest to find an effective antiobesity treatment.

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The survival of our species depends upon the drive to find food. Our ancestors were likely to have been those who were best able to survive famine by laying down fat in times of plenty, and conserving energy in times of food deprivation. Ironically, in present times these survival attributes make us particularly susceptible to the dangers of excess food. Obesity is a growing epidemic, causally associated with a number of serious medical conditions, including diabetes mellitus, coronary heart disease, and several cancers. Approximately 1000 people per week die prematurely in the UK because of obesity.

In recent years great advances have been made in our understanding of the peripheral signals that regulate appetite from the gut and adipose tissue, and how these act within the brain. In 1994, the adipocyte hormone leptin was discovered[1] which circulates at concentrations proportional to fat mass and inhibits food intake.[2] Leptin crosses the blood brain barrier to act via its receptor to inhibit orexigenic and stimulate anorexigenic neuropeptides in the arcuate nucleus (ARC) of the hypothalamus. Leptin is believed to play an important role in long term energy balance.

The gut hormones ghrelin and peptide YY (PYY), secreted from the gut in response to changes to nutritional status, also act on the ARC to regulate appetite. While food intake is stimulated by ghrelin, it is inhibited by PYY. In this article, the discovery, anatomy, and physiology of ghrelin and PYY will be discussed, with focus on the adaptive changes in diseases such as obesity and anorexia nervosa. Ghrelin and PYY are important therapeutic targets in the quest to find an effective antiobesity treatment.

The discovery of ghrelin was preceded by work with synthetic compounds, the growth hormone secretagogues (GHSs) which stimulate growth hormone (GH) release and increase food intake. The growth hormone secretagogue receptor (GHS-R) was identified as a G protein coupled receptor located in the hypothalamus and pituitary.[3] In 1999 an endogenous ligand for this orphan receptor was discovered, and purified from the stomach.[4] This ligand was named "ghrelin" from the Indo-European route *ghre* meaning to grow. Ghrelin has a biologically unique octanoyl fatty acid side chain, essential for its biological function, on the third of 28 linear amino acids.

PYY was initially isolated from colonic extracts as a 36 amino acid linear peptide in 1982,[5] with a tyrosine residue at both C and N terminals. It was after these two tyrosines (Y in peptide nomenclature) that PYY was named. Like ghrelin, the structure is highly preserved across species, suggesting that any significant mutation in PYY or ghrelin proved fatal to its owner. PYY shares considerable homology with pancreatic polypeptide (PP) from pancreatic endocrine cells and neuropeptide Y (NPY), a potent central orexigenic agent.[6] PYY, PP, and NPY may be considered a family of regulatory peptides. Five receptors have been identified within the brain for this family and named Y1–Y5 receptors.

Ghrelin is primarily secreted from X/A-like endocrine cells of the oxyntic glands of the stomach.[7] The majority of these cells abut the blood capillaries but do not have direct contact with the stomach lumen and gut nutrients. Ghrelin immunoreactivity is also found at lower concentrations within the hypothalamus,[4] an area of the brain known to be important in the regulation of appetite. PYY is secreted from the endocrine L cells of the small and large bowel, and is found at low concentrations throughout the small intestine, and at higher concentrations in the terminal ileum and colon with the maximum concentration in the rectum.[8] The major circulating form of PYY is PYY3–36.[9] PYY3–36 has been shown to be a selective agonist at the presynaptic inhibitory Y2 autoreceptor.[10]

Ghrelin levels are highest in the fasting state, rising sharply before, and falling within one hour of a meal.[11] Ghrelin peaks are of similar magnitude before each meal of the day. When all time cues were removed, ghrelin levels were found to rise when subjects requested a meal, suggesting that the actions of ghrelin on meal initiation are under neural control. In contrast with ghrelin,

Abbreviations: ARC, arcuate nucleus; PYY, peptide YY; GH, growth hormone; GHSs, growth hormone secretagogues; GHS-R, growth hormone secretagogue receptor; PP, pancreatic polypeptide; NPY, neuropeptide Y; Agrp, agouti related protein; POMC, proopiomelanocortin; BMI, body mass index.

PYY levels are suppressed in the fasting state, and increase following a meal. Plasma PYY levels rise within 30 minutes of nutrients reaching the gut,[12] despite the fact that PYY is secreted at the highest concentration in the colon, again suggesting neural regulation. PYY levels continue to rise for several hours after a meal by which time the nutrients may act directly on intestinal L cells. PYY levels are lowest in the morning and are elevated after breakfast, rise further after lunch and reach their daily peak a few hours after the evening meal.[13] Obese people tend to eat a larger proportion of their daily food intake in the evening.[14] This eating pattern could be associated with lower postprandial PYY levels and explain its association with an increased caloric intake.

In addition to its important role in the control of energy homeostasis, ghrelin stimulates GH secretion in pituitary culture cells and in vivo.[4] Peripheral ghrelin potently stimulates feeding in rodents, with the maximum effect being seen within one hour of administration.[15] A dose of ghrelin that stimulates feeding was found to achieve similar circulating levels as those observed after a 24 hour fast,[16] suggesting that ghrelin regulates day to day food intake. Chronic peripheral ghrelin administration leads to a significant increase in cumulative food intake and body weight gain with no attenuation in feeding stimulation with multiple injections.[16] This weight gain is mainly due to increased fat deposition,[17] and may be due to decreased energy expenditure[18] in addition to increased food intake. However, GH and insulin like growth factor 1 return to pretreatment levels during chronic ghrelin administration.[16] While ghrelin plays a long term role in the regulation of feeding and of body weight it may not chronically influence GH secretion.

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Peripheral administration of PYY was first reported to decrease appetite in 1993.[19] Recent work has demonstrated that PYY3–36, the major circulating form of PYY, inhibits appetite in the fasting state at physiological concentrations.[20] The finding that PYY3–36 has no effect on feeding in the Y2 receptor knockout mouse but inhibits feeding in wild-type litter mates supports the hypothesis that PYY modulates feeding through the Y2 receptor. Chronic administration of PYY3–36 leads to a decrease in food intake and a decrease in body weight. The effects of PYY3–36 on energy expenditure have yet to be investigated.

Human appetite is stimulated by ghrelin and inhibited by PYY3–36, both at physiological concentrations. Exogenous ghrelin infusion stimulated food intake from a free buffet meal by 28% in healthy volunteers and increased subjective hunger.[21] Despite the increased food intake, no change in satiety was reported, and cumulative food intake for that day was significantly more than on the saline control day. Conversely, exogenous PYY3–36 infusion decreased food intake from a free buffet meal by 36% and decreased subjective hunger. Cumulative food intake was reduced by 33% during the 24 hour period following PYY3–36 infusion.[20] Thus PYY plays a counterregulatory role to ghrelin in both the short and long term. No side effects were observed with either ghrelin or PYY infusions. These studies indicate that ghrelin, PYY, and their respective receptors are important potential therapeutic targets. Ghrelin treatment could increase appetite and lead to weight gain in patients such as those with cancer cachexia. Conversely, PYY3–36 or ghrelin blocking agents could provide novel treatments for obesity.

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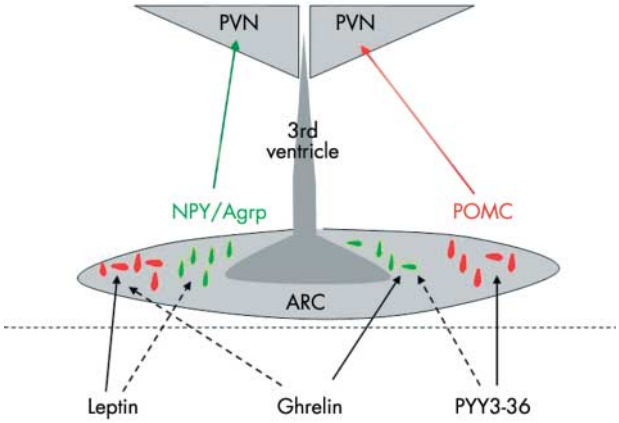

Figure 1 Hypothalamic actions of the peripheral hormones leptin, ghrelin, and peptide YY (PYY). ARC, arcuate nucleus; PVN, paraventricular nucleus; NPY, neuropeptide Y; Agrp, agouti related protein; POMC, proopiomelanocortin. Filled arrows indicate stimulatory action and broken arrows inhibitory action.

How is the nutrient status of the gut linked with the sensation of hunger within the mind? Peripheral ghrelin is thought to stimulate appetite through the ARC, an area of the hypothalamus known to be important in the regulation of feeding. After microinjection into defined hypothalamic sites, ghrelin was found to stimulate feeding most markedly in the ARC.[16] Moreover, peripheral ghrelin increases the number of cells expressing *c-fos* (an early gene product whose appearance is used as an index of neuronal activation) in the ARC. The majority of these *fos* positive neurones co-stain with NPY.[22] Ghrelin has also been shown to stimulate hypothalamic agouti related protein (Agrp) expression.[23] NPY and Agrp are in turn thought to stimulate feeding through receptors in the hypothalamic paraventricular nucleus[24] (fig 1).

Administration of the inhibitory gut hormone PYY3–36 also stimulates *c-fos* expression within the ARC but, like leptin and in contrast with ghrelin, inhibits hypothalamic NPY expression. PYY3–36 has been shown to increase the release of α-melanocyte concentrating hormone, a product of the anorexigenic CNS peptide proopiomelanocortin (POMC).[20] Therefore, PYY3–36 may exert its actions through stimulation of anorexigenic neurones such as POMC as well as inhibition of orexigenic neurones such as NPY.

What is the mechanism of the hypothalamic activation of peripheral ghrelin and PYY? Gut hormones may act directly on the ARC of the hypothalamus through semipermeable capillaries in the medium eminence.[25] Recently, it has been suggested that an intact vagus nerve is required for ghrelin to modulate feeding and GH release.[26] Following blockade of the gastric vagal afferent, peripheral ghrelin failed to stimulate feeding whereas centrally administered ghrelin stimulated feeding normally. Thus ghrelin from the stomach may act on the hypothalamus via the vagus nerve and brainstem. Further work is needed to determine whether PYY also stimulates brainstem neurones.

Circulating ghrelin levels are increased by up to threefold in states of undernutrition, such as anorexia nervosa.[27] Conversely, plasma ghrelin is decreased in obesity. As weight is lost, ghrelin levels rise whereas leptin levels fall[28] and vice versa. Changes in circulating ghrelin and leptin act to oppose weight change, and may explain why most people maintain a stable body weight despite marked variation in day to day food intake. For example, if weight is gained, ghrelin levels fall and leptin levels rise—changes which should reduce hunger and increase energy expenditure. Interestingly, PYY levels have been reported to be lower in obese people compared with lean

controls.[29] Thus in obesity, impaired inhibitory hunger signals from the gut may function as a positive feedback loop promoting further weight gain.

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Obesity may be thought of as a state of chronic adaptation to the hormonal changes of increased fat mass. In addition to increased plasma leptin and decreased ghrelin and PYY, there may also be up or downregulation in their hypothalamic receptor numbers. It remains to be seen whether obese people have altered sensitivity to ghrelin and PYY3–36. In evolutionary terms, avoidance of starvation is more important than protection against obesity. While the gastric oxyntic glands appear to be capable of producing ever increasing concentrations of ghrelin in states of undernutrition, could it be that the low levels of PYY in obesity are due to L cell failure? Perhaps the L cells of the intestine function similarly to the β cells of the pancreas and initially increase PYY secretion in response to increased food intake and weight gain. As further weight is gained, the body might become less sensitive to PYY, as it does to insulin, until the L cells fail to secrete enough PYY to provide the usual inhibition of appetite seen after a meal. If this were the case, antiobesity treatment in the early stages of weight gain might be most effective.

In this edition of *Gut,* Asakawa and colleagues[30] demonstrate for the first time the discovery of an effective ghrelin (GHS-R) antagonist, [D-Lys-3]-GHRP-6 **[see page 947]**. This agent has already been shown to inhibit the GH secretory effect of ghrelin.[31] Asakawa *et al* show that [D-Lys-3]-GHRP-6 inhibits feeding in the fasted state when endogenous ghrelin levels are high but has no effect on food intake in the fed state when circulating ghrelin levels are low.[30] Moreover, intracerebroventricular administration of [D-Lys-3]- GHRP-6 blocked the stimulatory feeding effect of peripheral ghrelin. In the leptin deficient *ob/ob* mouse, a naturally occurring rodent mutant model of obesity, chronic administration of [D-Lys-3]-GHRP-6 led to reduced body weight without affecting muscle mass. The authors conclude that these results may be due to antagonism of the actions of ghrelin at the GHS- R.[30]

Caution should be employed when assessing whether inhibition of food intake is due to true hormone blockade, or due to the toxic effects of the "antagonist" substance. For example, all of the effects described above could also be caused by a "poison" such as cyanide. In favour of [D-Lys-3]-GHRP-6 acting as a true antagonist is the behavioural study performed by Asakawa and colleagues.[30] There was no evidence of any difference in anxiety or of any gross behavioural change between animals treated with [D-Lys-3]-GHRP-6 compared with controls. It would be interesting to study whether this antagonist had any effect on neuropeptides that stimulate feeding independent of the ghrelin GHS-R receptor, such as galanin.

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Before [D-Lys-3]-GHRP-6 is considered for human use, the effects of its chronic administration in dietary models of obesity, such as rodents on a high fat diet, should be investigated. These findings are likely to be more applicable to the majority of obese humans who have increased plasma leptin than those from the leptin deficient *ob/ob* mouse. The challenge in developing any antiobesity drug is to find a treatment that selectively inhibits appetite without significantly affecting other neuroendocrine and gastrointestinal systems.

The only treatment which to date has been proven to achieve lasting weight reduction is gastric and intestinal bypass surgery. In one series, mean weight loss at 15 years post bypass surgery was 30 kg.[32] However, the morbidity and mortality associated with bypass surgery, in addition to practical and financial constraints, usually limit this approach to the severely obese patient (body mass index (BMI) >40 kg/m^2). Interestingly, the success of bypass surgery may be as much hormonal as mechanical as induced malabsorption is usually only temporary whereas the loss of appetite is permanent. Plasma ghrelin levels were recently measured in patients who had undergone gastric bypass.[28] Circulating ghrelin levels were 77% lower in the bypass group compared with BMI matched controls, and the usual pre-meal peaks were lost. After bypass surgery, whereas ghrelin levels are suppressed, there is significant elevation in PYY.[33] A recent study has shown that rats treated with bypass surgery have a threefold increase in circulating PYY with a 21% reduction in body weight after 28 days.[34]

Therefore, the success of bypass surgery is due, at least in part, to a decrease in circulating ghrelin and an increase in circulating PYY. These changes in gut hormones act on the arcuate nucleus of the hypothalamus, either directly through the median eminence or via the brainstem, to decrease expression of the orexigenic neuropeptides NPY and Agrp and increase expression of the anorexigenic neuropeptide POMC. Thus altered gut signals following bypass surgery act on the mind and may explain why bypass patients frequently describe amazingly reduced hunger following surgery.

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If obese people were sensitive to ghrelin and PYY, this would suggest that blocking ghrelin and augmenting PYY could provide an effective medical antiobesity strategy. These hormonal changes would mimic those seen after bypass surgery. Asakawa and colleagues[30] report important progress in this area with the development of a ghrelin antagonist. In the future, manipulation of the hormones of the gut might reduce hunger in the mind.

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REFERENCES

1 **Zhang Y**, Proenca R, Maffei M, *et al*. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;**372**:425–32.
2 **Friedman JM**, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998;**395**:763–70.
3 **Howard AD**, Feighner SD, Cully DF, *et al*. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 1996;**273**:974–7.
4 **Kojima M**, Hosoda H, Date Y, *et al*. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999;**402**:656–60.
5 **Tatemoto K**, Mutt V. Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. *Nature* 1980;**285**:417–8.
6 **Tatemoto K**, Carlquist M, Mutt V. Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* 1982;**296**:659–60.

7 **Date Y**, Kojima M, Hosoda H, *et al*. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000;**141**:4255–61.
8 **Adrian TE**, Ferri GL, Bacarese-Hamilton AJ, *et al*. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 1985;**89**:1070–7.
9 **Grandt D**, Schimiczek M, Beglinger C, *et al*. Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1–36 and PYY 3–36. *Regul Pept* 1994;**51**:151–9.
10 **Keire DA**, Mannon P, Kobayashi M, *et al*. Primary structures of PYY, [Pro(34)]PYY, and PYY-(3–36) confer different conformations and receptor selectivity. *Am J Physiol Gastrointest Liver Physiol* 2000;**279**:G126–31.
11 **Cummings DE**, Purnell JQ, Frayo RS, *et al*. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001;**50**:1714–9.
12 **Anini Y**, Fu-Cheng X, Cuber JC, *et al*. Comparison of the postprandial release of peptide YY and proglucagon-derived peptides in the rat. *Pflugers Arch* 1999;**438**:299–306.
13 **Soffer EE**, Adrian TE, Launspach J, *et al*. Meal-induced secretion of gastrointestinal regulatory peptides is not affected by sleep. *Neurogastroenterol Motil* 1997;**9**:7–12.
14 **Berteus FH**, Lindroos AK, Sjostrom L, *et al*. Meal patterns and obesity in Swedish women—a simple instrument describing usual meal types, frequency and temporal distribution. *Eur J Clin Nutr* 2002;**56**:740–7.
15 **Wren AM**, Small CJ, Ward HL, *et al*. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000;**141**:4325–8.
16 **Wren AM**, Small CJ, Abbott CR, *et al*. Ghrelin causes hyperphagia and obesity in rats. *Diabetes* 2001;**50**:2540–7.
17 **Tschop M**, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000;**407**:908–13.
18 **Asakawa A**, Inui A, Kaga T, *et al*. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001;**120**:337–45.
19 **Okada S**, Ohshima K, Mori M, *et al*. Peripheral not central administered PYY decreases high fat diet intake. *Endocrinology* 1993;(Suppl):180.
20 **Batterham RL**, Cowley MA, Small CJ, *et al*. Gut hormone PYY(3–36) physiologically inhibits food intake. *Nature* 2002;**418**:650–4.
21 **Wren AM**, Seal LJ, Cohen MA, *et al*. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001;**86**:5992.
22 **Wang L**, Saint-Pierre DH, Tache Y. Peripheral ghrelin selectively increases Fos expression in neuropeptide Y—synthesizing neurons in mouse hypothalamic arcuate nucleus. *Neurosci Lett* 2002;**325**:47–51.
23 **Kamegai J**, Tamura H, Shimizu T, *et al*. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology* 2000;**141**:4797–800.
24 **Broberger C**, Johansen J, Johansson C, *et al*. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proc Natl Acad Sci U S A* 1998;**95**:15043–8.
25 **Cone RD**, Cowley MA, Butler AA, *et al*. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *Int J Obes Relat Metab Disord* 2001;**25**(suppl 5):S63–7.
26 **Date Y**, Murakami N, Toshinai K, *et al*. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 2002;**123**:1120–8.
27 **Ariyasu H**, Takaya K, Tagami T, *et al*. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab* 2001;**86**:4753–8.
28 **Cummings DE**, Weigle DS, Frayo RS, *et al*. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* 2002;**346**:1623–30.
29 **Alvarez BM**, Borque M, Martinez-Sarmiento J, *et al*. Peptide YY secretion in morbidly obese patients before and after vertical banded gastroplasty. *Obes Surg* 2002;**12**:324–7.
30 **Asakawa A**, Inui A, Kaga T, *et al*. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut* 2003;**52**:947–52.
31 **Pinilla L**, Barreiro ML, Tena-Sempere M, *et al*. Role of ghrelin in the control of growth hormone secretion in prepubertal rats: interactions with excitatory amino acids. *Neuroendocrinology* 2003;**77**:83–90.
32 **Mitchell JE**, Lancaster KL, Burgard MA, *et al*. Long-term follow-up of patients' status after gastric bypass. *Obes Surg* 2001;**11**:464–8.
33 **Naslund E**, Gryback P, Hellstrom PM, *et al*. Gastrointestinal hormones and gastric emptying 20 years after jejunoileal bypass for massive obesity. *Int J Obes Relat Metab Disord* 1997;**21**:387–92.
34 **le Roux CW**, Shurey S, Ghatei MA, *et al*. PYY and decreased appetite following jejunum intestinal bypass in rats. *British Endocrine Society*, Glasgow, 2003:endocrine abstracts.